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Impaired NK cell functionality and increased TNF-α production as biomarkers of chronic chikungunya arthritis and rheumatoid arthritis

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ABSTRACT

The chronic chikungunya arthritis symptoms closely mimic the rheumatoid arthritis (RA) symptoms, thus making it difficult to distinguish between these two clinical entities. The current comparative study characterizes NK ($CD3^-CD56^+$) and NK-like T ($CD3^+CD56^+$) cell responses in patients with chronic chikungunya arthritis and RA. Phenotype and functions of NK and NK-like T cells repertoire were assessed in 56 chronic chikungunya arthritis, 26 RA patients and 82 controls using flow cytometry. TNF-α and IFN-γ-secreting NK-like T cells were high in both chronic arthritis patients than in controls. Percentage of TNF-α^+ NK cells was higher in RA patients than in controls. Percentage of perforin$^+$ NK cells was low in both chronic arthritis patient groups. Among the patient groups, expressions of perforin$^+$ and IFN-γ^+ NK-like T cells were higher in RA. Overall, our data show reduced frequency of NK-like T cells, lower expression of perforin$^+$ NK, higher expression of TNF-α^+ NK-like T and IFN-γ^+ NK-like T cells as the markers of chronic arthritic diseases. In the absence of any specific treatment for chronic chikungunya induced arthritis and promising results of anti-TNF-α therapy against RA, current data may form the basis for future *in vivo* studies and has scope as possible therapeutics against chikungunya.

1. Introduction

Chikungunya virus (CHIKV), transmitted by the *Aedes* mosquito has caused several epidemics throughout the world [1,2]. The illnesses characterized by high-grade fever, rashes, myalgia and severe joints pain mainly affecting the extremities that mostly resolve within the acute phase [1,3]. However in some patients, joint pain and/or swelling can persist for months or years following the initial infection, which is the hallmark of the chronic phase of chikungunya [4]. Following acute phase, 40–80% of patients develop unresolved rheumatic manifestations associated with inflammation and musculoskeletal tissue destruction [5].

Genetic and environmental factors interact and contribute to the development of autoimmune diseases including rheumatoid arthritis (RA) [6]. RA is a chronic immune inflammatory disease associated with inflammation of the synovial membrane that leads to the destruction of cartilage and bones [7]. It causes severe pain, swelling, stiffness in the joints and may lead to joint deformity [8]. The chronic chikungunya symptoms closely mimic those of RA, thus, it may be difficult to distinguish between these two clinical situations. This is a unique challenge before the rheumatologists in designing differential diagnosis for chronic polyarthritis. Chikungunya arthropathy is crippling and may be long lasting. Though similar looking clinically, both conditions differ in serological parameters [RA factor, cyclic citrullinated peptide antibody (anti-CCP antibody)]. However, Joint pain induced in both arthritis of chikungunya and RA has a negative impact on everyday activities.

Therapeutic plasmapheresis or depleting B cells with the antibody rituximab are reported to be beneficial in RA. Anti-TNF-α antibody or sTNF-R-Fc fusion protein have also been approved for treating RA [9,10]. No specific anti viral treatment is available for chikungunya. Cytokine analysis of these patients may provide the rationale for their use for treatment. Chikungunya patients with persistent prolonged arthralgias respond better to chloroquine [11]. Chloroquine phosphate offers symptomatic relief and also may act as antiviral agent to combat chikungunya virus [12]. Disease modifying drugs like methotrexate and sulfasalazine and

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chloroquin/hydroxyl chloroquineare useful for management of chronic chikungunya arthritis [11].

In RA, the innate immune system is persistently activated and its detailed understanding had led to enticing targets for new therapeutic interventions [13]. A key role of the innate immune response in virus suppression, propagation, and dissemination is well established. Prime cells of the innate immune response, natural killer (NK) and natural killer T (NKT) cells can kill target cells directly or interact with antigen-presenting cells and T cells to produce cytokines having antiviral activities [14].

Infiltration of NK cells into the synovial fluid has been reported in patients suffering from chronic chikungunya and RA [15,16]. Studies on humans and non-human primates have shown the participation of NK cells in the early control of CHIKV [17,18]. In a study in early acute patients, Petitdemange et al. [19] have reported that NK cells sense CHIKV from the beginning of infection and may thus contribute to viral clearance and in a similar line, our group has shown that NK ($CD3^-CD56^+$)/NK-like T ($CD3^+CD56^+$) cells can deliver an early and efficient immune response following CHIKV infection [20]. Deregulated NK/NK-like T cells functionality have been associated with chronic chikungunya arthritis (our unpublished data). Currently, understanding of immunologic and virologic mechanism which makes a broad range of patient groups spanning from asymptomatic to persistent, debilitating arthritis is limited. Thus, the phenotypic and functional study of immune cells in the CHIKV induced arthritis and RA patients could provide insights into the chronic polyarthritis diseases as well. The current study evaluates the phenotype and functions of NK ($CD56^+CD3^-$) and NK-like T ($CD56^+CD3^+$) cells repertoire in patients with chronic chikungunya arthritis and RA.

2. Materials and methods

2.1. Study population

This study was approved by the Institutional Ethical Committee for Research on Humans, as per the guidelines set by the Indian Council of Medical Research, New Delhi, India. Informed written consent was obtained from all participants.

The patient group comprised of 56 chronic chikungunya arthritis patients and 26 RA patients without chikungunya. The patients were enrolled from Sanjeevan Hospital, Latur. All the patients included in the current study were treatment naïve. Eighty two apparently healthy individuals from the blood donation camps organized in Pune were recruited as controls (Table 1).

Inclusion criteria for enrolment were [1] *chronic chikungunya arthritis patients:* chronic persistent inflammatory polyarthritis/arthralgias lasting for more than three months following an episode of acute febrile polyarthritis and positive for chikungunya IgM/IgG antibodies [11,21]. Exclusion criteria for this group were the presence of RA factor, anti-CCP antibody, ANA positivity and raised serum uric acid levels. The disease affects all age groups with female preponderance. Disease onset is with acute febrile polyarthritis with/without rash. In the chronic phase, patients have inflammatory symmetric polyarthritis affecting small and large joints of hands and feet with rheumatoid distribution. [2] *RA patients:* chronic inflammatory disorder of unknown etiology (characterized by symmetric polyarthritis, constitutional features and sometimes multisystem involvement) with symptom duration for more than 6 weeks. The patients were classified as RA according to 2010 ACR/EULAR classification criteria for RA [22]. Exclusion criteria for this group were positivity for ANA, raised serum uric acid, positivity for anti-CHIKV IgG and IgM antibodies. [3] *Controls:* negative for anti-CHIKV IgM and IgG antibodies, RA factor, anti-CCP antibody and ANA with normal serum uric acid.

All samples were screened by ELISA for antibodies against dengue virus [21] and only samples negative for dengue virus were included in the study.

2.2. Flow cytometric analysis

2.2.1. NK/NK-like T cells enumeration

Whole blood staining was carried out from freshly drawn blood samples of 56 chronic chikungunya patients with arthritis, 26 RA patients and 82 controls using appropriate monoclonal antibodies and following the staining protocol used for NK and NK-like T cells as described earlier[20,23]. The gating strategy is shown in Supplementary Fig. 1.

2.2.2. Degranulation assay, perforin assay and Intracellular cytokine staining

Degranulation activity based on anti-CD107a (Lamp1) expression, expression of IFN-γ, TNF-α and perforin were assessed on NK/NK-like T cells in 10 samples from each study group following the previously reported protocol [20]. The representative flow cytometry plots are shown in Supplementary Fig. 1.

2.3. Software and statistical analysis

Statistical analyses were performed using the SPSS 23 software (SPSS Inc., IL, USA). Intergroup comparisons were assessed using a nonparametric Mann–Whitney *U* test. The data was expressed as mean (range). $p < 0.05$ were considered statistically significant. The results were discussed only when the RA group was statistically different from either one or both study groups.

3. Results

3.1. NK and NK-like T cells in chronic chikungunya arthritis and RA patients

To assess the status of innate immune cell phenotypes in chronic chikungunya arthritis in comparison to RA and control, NK and NK-like T cell frequencies were measured by flow cytometry. NK cell percentage was lower in RA patients than in chronic chikungunya arthritis patients and controls [RA: 2 (0.2–8.3) vs. chronic chikungunya arthritis: 5.7 (0.1–16.9), and controls: 7.8 (1.4–37.6), $p < 0.0001$ in each case] (Fig. 1A). Percentages of

Table 1
Characteristics of the study population.

Parameters	Chronic arthritis chikungunya	RA	Controls
Study population	n = 56	n = 26	n = 82
Gender ratio (Male: Female)	0.2	0.5	1.2
Age (years): mean (range)	39 (12–65)	50 (19–65)	40(21–62)
Post infection months: mean (range)	86 (3–109)	NA	NA
Anti-CHIKV IgM	Positive/Negative	Negative	Negative
Anti-CHIKV IgG	Positive	Negative	Negative

NA: not applicable.